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**Commentary: Lipid Transport in *Mycobacterium tuberculosis* and its
Implications in Virulence and Drug Development**

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ABSTRACT

Tuberculosis is still a major health problem worldwide and one of the main causes of death by a single infectious agent. Only few drugs are really effective to treat tuberculosis, hence, the emergence of multiple, extensively, and totally drug resistant bacilli compromises the already difficult antituberculosis treatments. Given the persistent global burden of tuberculosis, it is crucial to understand the underlying mechanisms required for the pathogenicity of *Mycobacterium tuberculosis* (Mtb), the causal agent of tuberculosis, in order to pave the way for developing better drugs and strategies to treat and prevent tuberculosis.

The exclusive mycobacterial cell wall lipids such as trehalose monomycolate and dimycolate (TMM, TDM), phthiocerol dimycocerosate (PDIM), sulpholipid-1 (SL-1), diacyl trehalose (DAT), and pentacyl trehalose (PAT), among others, are known to play an important role in pathogenesis; thus, proteins responsible for their transport are potential virulence factors. MmpL and MmpS proteins mediate transport of important cell wall lipids across the mycobacterial membrane. In Mtb, MmpL3, MmpL7 and MmpL8 transport TMM, PDIM and SL-1 respectively. The translocation of DAT and biosynthesis of PAT is likely due to MmpL10. MmpL and MmpS proteins are involved in other processes such as drug efflux (MmpL5 and MmpL7), siderophore export (MmpL4/MmpS4 and MmpL5/MmpS5), and heme uptake (MmpL3 and MmpL11). Altogether, these proteins can be regarded as new potential targets for antituberculosis drug development. We will review recent advances in developing inhibitors of MmpL proteins, in the challenging context of targeting membrane proteins and the future prospects for potential antituberculosis drug candidates.

Keywords

tuberculosis, lipid transport, MmpL/S proteins, drug resistance, transport proteins

Tuberculosis along history

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, has been present in the human population since antiquity. Initially, it was thought that during the domestication of animals in the Neolithic period, *Mycobacterium bovis* strains from infected cattle evolved and acquired the capability of infecting humans, hence originating human tuberculosis [1]. However, it is now widely accepted that the ancient Mtb strains were originated from environmental mycobacteria 70 000 years ago in Africa [2, 3]. The introduction of agriculture, civilization and the increase in human population density led to the selection of virulent and transmissible Mtb strains. These modern Mtb strains spread throughout the world causing the tuberculosis epidemics that ravaged mankind for centuries [4].

Nowadays, 9 million people fell ill with tuberculosis and 1.5 million died in 2013 (including 360 000 deaths among HIV-positive people) according to the most recent report of the World Health Organization (WHO) [5]. At present, tuberculosis is the second leading cause of death from an infectious agent worldwide, after the Human Immunodeficiency Virus (HIV), in spite of the fact that we have powerful tools in order to face tuberculosis: vaccination, diagnostics and treatments.

Tuberculosis treatment and drug resistance

Drug treatment is the only effective therapy for tuberculosis. Drugs are classified into three groups (Table 1) based on evidence of efficacy, potency and experience of use [6].

- First-line antituberculosis drugs are the most effective and widely used drugs for the treatment of drug-susceptible tuberculosis.
- Second-line antituberculosis drugs are reserved for treating bacilli resistant to first-line therapy. A drug may be classed as second-line instead of first-line for being less effective than the first-line drugs (e.g. para-aminosalicylic acid), having toxic side-effects (e.g. cycloserine) or being effective, but unavailable in many developing countries (e.g. fluoroquinolones).
- Third-line antituberculosis drugs are characterized for being less effective (e.g. clarithromycin) or because their efficacy has not been fully proven (e.g. clofazimine).

With appropriate antibiotic treatment, around 90% of HIV-negative patients with drug-susceptible tuberculosis can be cured in 6 months using a combination of rifampicin (Rif), isoniazid (Inh), pyrazinamide (Pza) and ethambutol (Emb) for 2 months, followed by a four-month continuation phase of Rif and Inh [5]. The main reason for prescribing this combination of medicines in the treatment of tuberculosis is because the likelihood of the emergence of multiple drug resistance bacteria is virtually impossible [7], apart from the fact that the distinct antituberculosis drugs have different modes of action: Inh are bactericidal against replicating bacteria; Emb is

bacteriostatic at low doses, but is used in tuberculosis treatment at higher, bactericidal doses; Rif is bactericidal and has a sterilizing effect; and Pza is only weakly bactericidal, but is very effective against bacteria located in acidic environments, inside macrophages, or in areas of acute inflammation, where they enter into a non-replicative condition. Thus, the combination of all these drugs targets all subpopulations of Mtb, i.e. those actively replicating and those in a non-replicative state.

Monotherapy, irregular drug supply, poor drug quality, inappropriate prescription, poor adherence to treatment and unsuitable supervision and support on the part of health personnel, can contribute to appearance and selection of drug-resistant Mtb strains that can disseminate and cause drug resistant tuberculosis. Depending on the drug resistance pattern, three major categories have been defined:

- Multidrug-resistant (MDR) strains are those resistant to Inh and Rif. In 2013, the highest levels of MDR tuberculosis were found in Eastern Europe and central Asia, where in some countries more than 20% of new tuberculosis cases and more than 50% of those previously treated for tuberculosis have MDR tuberculosis [5].
- Extensively drug-resistant (XDR) strains are MDR strains (i.e, resistant to Inh and Rif) that are also resistant to a quinolone and one of the second-line injectable drugs (kanamycin, amikacin or capreomycin). In 2006, the first XDR tuberculosis outbreak was described in KwaZulu-Natal in South Africa. The mortality rate among HIV-positive patients, with limited or no access to highly

active antiretroviral therapy was 98%, after a median survival period from diagnosis of only 16 days [8].

- Totally drug-resistant (TDR) refers to strains that are resistant to all available tuberculosis drugs, although the number and level of resistance to each drug has not yet been precisely defined. To date, only a limited number of TDR tuberculosis cases have been confirmed in Iran, India, South Africa and Italy [9].

All these are cases of acquired resistance [7]. The mechanisms by which bacteria in general acquired drug resistance are: barrier mechanisms (decreased permeability/increased efflux), degrading/inactivating enzymes, modification of pathways involved in drug activation/metabolism, and drug target modification (mutations) or target amplification [10]. *Mtb* in particular is able to acquire drug resistance by spontaneous mutations in chromosomal genes then leading to target modification, target amplification, reduced ability for activating drugs, or increased capacity for inactivating drugs (Table 2); no horizontal transfer of resistance genes had been reported [11].

Mycobacterial cell envelope: biochemically complex, pharmacologically interesting

The mycobacterial envelope is unique, both in molecular composition and in the architectural arrangement of its constituents (Fig. 1; adapted from [12, 13]). Its complex structure is composed of a typical phospholipid bilayer plasma membrane, an outer membrane called mycomembrane, and an outermost layer known as the

capsule. The plasma membrane is composed mainly of anionic phospholipids in a bilayer arrangement with proteins. The mycomembrane consists of an asymmetric lipid bilayer made of long chain (C60-C90) mycolic fatty acids in the inner leaflet, and free intercalating glycolipids and waxy components on the outer leaflet. Cryo-electron microscopy images support a folded or compact configuration of these mycolic chains, which reminiscent of Gram-negative bacterial cell walls [14-16], and also confirm that the measured thickness of the outer membrane is consistent with the size of mycobacterial porins, such as MspA from *M. smegmatis*, which may therefore form channels in this bilayer [14]. The outer and inner membrane form a periplasmic space, with the presence of a thin layer of peptidoglycan covalently linked to arabinogalactan and lipoarabinomannan, which in turn are bound to mycolic acids. Peptidoglycan, arabinogalactan and mycolic acids form the cell wall skeleton. The capsule is mainly composed of polysaccharides, proteins and small amounts of lipids, and it is considered to have a different molecular composition in pathogenic and non-pathogenic species [17]. The outermost layer is visible in conventional electron microscopy preparations only when cultures have been grown in free detergent medium. It is thought that mycobacterial growth under laboratory routine culturing conditions (with a detergent as Tween-80) promotes removing of this layer [13].

The mycobacterial envelope is involved in important roles, such as defining the shape of the cell and providing mechanical and osmotic protection. Its unusual high hydrophobicity makes it an efficient barrier for chemotherapeutic agents. Other important function is the transport of molecules, including nutrients, ions and toxic metabolites. During infection, cell-wall compounds have been shown to trigger a set

of biological effects including adjuvanticity, toxicity, immune down-modulation, and arrest of phagosome maturation [18]. For these reasons, antitubercular drugs acting by inhibiting biosynthesis or assembly of mycobacterial cell envelope components are very effective antituberculosis drugs. This is the case of Inh, one of the two major drugs in tuberculosis treatment, and ethionamide: both are prodrugs that need activation by catalase-peroxidase (KatG) and mono-oxygenase (EthA), and their active products inhibit InhA, an enzyme involved in mycolic acids biosynthesis; similarly, Emb directly blocks biosynthesis of arabinan and its assembly into the bacterial arabinogalactan. Since these three drugs (Inh, ethionamide, and Emb) inhibit biosynthesis of cell wall components that are exclusive of mycobacteria and a few related genera, these drugs are extremely specific for mycobacterial pathogens, having little (if any) activity against other bacterial pathogens.

In addition to mycolic acids, there are many other lipids that are also very important components of the mycobacterial cell wall, most of them being exclusive for mycobacteria. Among them can be found trehalose dimycolate (TDM), often referred as cord factor, trehalose monomycolate (TMM), glucose monomycolate (GMM), glycerol monomycolate, diacyl trehaloses (DAT), triacyl trehaloses (TAT), pentacyl trehaloses (PAT), the recently characterized family of mannosyl- β -1-phosphomycoketides, sulpholipids (SLs), phenolic glycolipids (PGLs) and phthiocerol dimycocerosate (PDIM). Other major glycolipids are lipomannan (LM), lipoarabinomannan (LAM) and phosphatidylinositol mannosides (PIMs). The importance of lipids for mycobacteria must be regarded from a double point of view: on the one hand, they contribute to the structure of the mycobacterial cell envelope, and in addition, many lipids have important roles in pathogenesis. A well-known

example of this is PDIM, which is found only in pathogenic mycobacteria; PDIM is essential during the early step of infection when bacilli encounter their host macrophages. In fact, mutant *Mtb* strains defective in genes encoding enzymes that take part in biosynthesis of PDIM (such as FadD26, FadD28 enzymes in cooperation with polyketide synthases) or in PDIM transport (such as DrrC protein, which forms an ABC transporter along with DrrB and DrrC) showed attenuated phenotypes in mice [19, 20]. Similarly, PIMs and mannose-capped lipoarabinomannan (Man-LAM) both interfere with phagosome maturation [21].

In summary, proper localization of lipids in the mycobacterial cell wall, mostly in the outermost layer, is needed for their role in pathogenicity, thus making transport of lipids an essential process in mycobacterial cells. Proteins transporting lipids are therefore important virulence factors and attractive drug targets.

Proteins for lipid transport in *M. tuberculosis*

Efflux pumps are membrane proteins that transport actively a wide variety of compounds across bacterial envelope. They have been classified into five superfamilies: ATP-binding cassette (ABC), major facilitator super-family (MFS), resistance nodulation division (RND), small multidrug resistance (SMR) and multidrug and toxic-compound extrusion (MATE). While MFS, SMR, RND and MATE members are secondary transporters, typically energized by the proton motive force (H^+ or Na^+), members of the ABC superfamily use ATP as the energy source and are considered as primary transporters [22]. Whereas efflux pumps are mostly known because of the transport of drugs from the cytoplasm, other efflux pump substrates

are sugars, lipids, proteins, synthetic compounds, toxic metabolites, host-defence molecules, virulence factors, etc. Such a heterogeneous substrate profile allows bacterial efflux pumps to play diverse roles in drug resistance, virulence, bacterial cell physiology, and detoxification [22], among others.

Mce proteins are ABC transporters implicated in virulence. The Mce proteins are encoded by the *mce1*, *mce2*, *mce3* and *mce4* operons in the genome of Mtb. The involvement of Mce4 transport system in cholesterol import and intracellular survival has been confirmed [23, 24], but the role of *mce1*, *mce2* and *mce3* operons is not clearly established, especially in the case of the *mce3* operon. As regards to *mce1* operon, it has been suggested that these proteins may serve as a mycolic acid re-importer [25], and *mce2* operon might be related in sulpholipid transport [26]. Due to their most probable implication in lipid metabolism, the Mce proteins may modulate pathogenicity through changes in Mtb lipid pathways.

A special case for lipid transport: the MmpL and MmpS families of proteins

The transport of lipids in Mtb is predominantly via a family of proteins termed MmpL proteins (Mycobacterial Membrane Protein Large) belonging to the RND family of membrane proteins. The Mtb genome possesses 15 different genes encoding for RND proteins [27], 13 of which belong to MmpL (Table 3) protein family. Mutants with disruptions in *mmpL2*, *mmpL4*, *mmpL5*, *mmpL7*, *mmpL8*, *mmpL10*, and *mmpL11* showed significant attenuation for growth in mice lungs [20, 28-30]. Notably, the first Mtb genome also revealed that many clusters harbouring polyketide synthase genes with a known or putative role in lipid biosynthesis also contained an *mmpL* gene

suggesting that the function of the *mmpL* gene was associated with the cognate lipid species produced by the enzymes encoded by the cluster. The first role for MmpLs in lipid transport was demonstrated by Cox *et al* [31] who isolated an *mmpL7* mutant from a signature-tag mutagenesis screen. The mutant was found to be defective in PDIM transport and found to accumulate the complex lipid intracellularly [31]. *mmpL7* is present in the same cluster that encodes enzymes responsible for the biosynthesis of the pthiocerol (*ppsA-E*) and mycocerosic acid (*mas*) moieties of PDIM. Subsequently, another MmpL protein, MmpL8 was shown to be involved in the transport of SLs [29, 32]. Interestingly, while no sulpholipid-1 (SL-1) was detected in the cell envelope of Mtb, the *mmpL8* mutant accumulated an intermediate of SL-1, termed SL-1278, indicating that some, if not all MmpLs were involved in transporting intermediates, which were consequently processed further outside the cell [29, 32].

More recently, the functions of other *mmpL* genes have been deciphered. MmpL10 was shown to be involved in the translocation of acylated trehaloses; loss of *mmpL10* function led to intracellular accumulation of DATs [33]. Additionally, PATs were missing from the cell surface, indicating that the translocation of DATs by MmpL10 was likely required for the subsequent acylation of DAT substrates to yield extracellular PATs. MmpL proteins are also involved in the transport of mycolic acid derived lipids. *mmpL3*, the only essential *mmpL* gene in Mtb was shown to be involved in the transport of TMM [34, 35] using a conditional mutant of the *mmpL3* homologue of *Mycobacterium smegmatis*. Furthermore, *mmpL11*, located in the same cluster as *mmpL3* was shown to be involved in the export of monomeromycolyl diacylglycerol (MMDAG) [36].

There is also a growing line of evidence that suggests the MmpLs are not just transporters, but may be involved in the formation of membrane-associated scaffolds that facilitate the coupling of lipid biosynthesis with transport. Four *mmpL* containing clusters in Mtb also contain a gene encoding an MmpS protein (Mycobacterial Membrane Protein Small). In *M. smegmatis*, MmpS4 was shown to be required for biosynthesis and export of a surface exposed glycopeptidolipid [37]. A domain of MmpL7 was shown to interact PpsE, an enzyme involved in PDIM biosynthesis [38]. MmpL3 was also shown to co-localise with meromycolate producing FAS-II complex components at the septa and poles of the mycobacterial cell [39].

Other roles for MmpL and MmpS proteins in *M. tuberculosis*

Besides lipid transport, MmpL and MmpS proteins are involved in other bacterial processes, including drug resistance and siderophore export [40] (MmpL4/MmpS4 and MmpL5/MmpS5), and heme uptake [41, 42] (MmpL3 and MmpL11); the acquisition of iron is an essential attribute of pathogenic bacteria so as to establish a successful infection.

a) Drug resistance

Early studies suggested that MmpL proteins did not play a significant role in intrinsic resistance to drugs in Mtb [30]; although this seems to be the general case, a few examples have been reported recently involving MmpL proteins in resistance to certain drugs. The MmpL5-MmpS5 proteins were originally characterised for their contribution to drug resistance in Mtb, which was mediated by active efflux of

econazole and other substrates from cells [43]. In recent years, these proteins were linked to cross-resistance between clofazimine (a traditional drug to treat leprosy, which is also considered as a third-line drug for use against drug resistant tuberculosis) and bedaquiline (a recently approved drug for treatment of MDR tuberculosis) [44, 45]. In all cases, drug resistance was mediated by increased transcription of these two genes, mediated by mutations in a regulatory protein encoded by Rv0678 gene; the latter could probably regulate as well expression of *mmpL4-mmpS4* and *mmpL2 –mmpS2* [46].

Evidences about a role for MmpL7 transporter in isoniazid resistance are still controversial: isoniazid readily increases transcription of *mmpL7* gene in Mtb [47], although overexpression of *mmpL7* gene only resulted in resistance to isoniazid in the heterologous host *M. smegmatis* [48].

The link between MmpL3 and drug resistance seems to be indirect and has not been fully characterised yet. MmpL3 interacts with Wag31 protein as a part of a large network of interactions between proteins involved in fatty acid metabolism; as a consequence of Wag31 knock-down, resistance to lipophilic drugs was altered [49].

b) Iron acquisition

Mtb has developed two strategies for acquiring iron when infecting the human host, and MmpL proteins play an important role in both pathways. First, a secreted protein, Rv0203, binds heme groups (an abundant iron source in mammalian tissues and proteins) in the outside of the bacteria, and then transfer it to MmpL3 and MmpL11

proteins that will transport iron-containing heme groups through the mycobacterial membrane into the cytoplasm, where MhuD protein will degrade heme group releasing its iron content [41]. Second strategy consists on producing and exporting siderophores (mycobactins and carboxymycobactins) that will bind non-heme iron outside the bacterial cell. MmpS4 and MmpS5 proteins resulted essential for producing and releasing siderophores, hence indicating a role for MmpL4-MmpS4 and MmpL5-MmpS5 protein complexes in exporting siderophores in Mtb [40]. Iron-containing siderophores are subsequently uptaken by a pathway independent of MmpL4-MmpS4 and MmpL5-MmpS5, which complete the siderophore recycling system; in those mutants lacking MmpS4 and MmpS5 proteins, siderophores then accumulate in the cytoplasm resulting in lethality for the bacteria [50].

Inhibitors of MmpL proteins: new drugs for the future?

MmpL proteins have been investigated for their contribution to virulence, drug resistance, and other processes in Mtb for more than a decade, but it was just in the last three years when MmpL3 (that had been predicted to be the only essential protein of this family [30]) emerged as a drug target. From 2012, several families of structurally different chemical entities have been shown to inhibit MmpL3 (Table 4) opening a new field in the inhibition of mycolic acids transport [34, 51-62]. Notably, compound SQ109, which was identified a decade ago in a combinatorial chemistry-based search for ethambutol analogues, currently on phase III of clinical trials, was revealed as an inhibitor of MmpL3 [54].

Discovering a brand new drug target in Mtb along with 7 new families of potential inhibitory compounds in such a short period of time seemed like a success in antituberculosis drug discovery never before seen. Further investigation into their mechanism of action demonstrated that most of these compounds are also targeting several enzymes in the menaquinone biosynthesis pathway and electron transport chain; as a consequence, respiration and ATP synthesis were inhibited and the transmembrane electrochemical proton gradient (the energy source in many transport processes) were abolished [63, 64]. Then, since multiple protein targets can be affected simultaneously, the probability of selection of resistant mutants remains very low for most of these compounds. Also, given that the electrochemical gradient, a general process in many cells, is targeted, compounds in this group such as SQ109, BM212 [55, 58] and the THPPs [60] have shown to be active against other bacterial and fungal pathogens lacking mycolic acids and MmpL3-related targets. In any case, the activity of these compounds against drug resistant and MDR isolates of Mtb and their synergy with current antituberculosis therapy (notably in the case of SQ109) makes conceivable the possibility to shorten current antituberculosis treatment, one of the key points that will be essential in the near future to control this terrible disease.

Future directions: the impact of MmpL proteins & lipid transport in antituberculosis drug discovery

For decades, the role of transport proteins in drug resistance in Mtb was considered to be marginal. While most of resistant strains, isolated from clinical specimens or selected under laboratory conditions, carried mutations in genes encoding target

proteins or activating enzymes, concerning efflux pumps the situation was far distant to that in Gram-negative bacteria, where efflux-mediated drug resistance is prominent in many pathogens [65].

Being enthusiastic about the role of transport proteins in drug resistance, we suggested that efflux pumps could have a role in transporting new drugs in development, and hence they should be taken into account in antituberculosis drug discovery programmes [66]. Recently, several publications have confirmed this hypothesis, and transport proteins were genetically related with resistance to peptidoglycan synthesis inhibitors [67] and several other families of new compounds with antituberculosis activity [68, 69]. Notably, among a series of spectinomycin derivatives, the most active molecules were those that escaped transport by the Rv1258c efflux pump [70]. Similarly, this has been the case for the MmpL5 protein since it has been demonstrated to transport bedaquiline [44, 45]. Altogether, it becomes clear that efflux transporters must be taken into consideration in any tuberculosis drug discovery programs in order to estimate the likelihood of the new molecules for being transported out of bacterial cells.

One of the most successful approaches in antimicrobial therapy has been the combination of beta-lactam antibiotics with inhibitors of beta-lactamases, and this has inspired a similar strategy for drug transport proteins [66, 71]. For years, this approach seemed unrealistic, given the toxicity of most efflux inhibitors, which remained useful only under laboratory conditions to demonstrate efflux mechanism. However, discovery of several MmpL3 inhibitors on recent years has brought the attention back to this field. Being a transport protein of the RND superfamily, MmpL3

is however rather distinct from conventional efflux pumps of the same family such as AcrAB-TolC from *E. coli*, in terms of substrate specificity and transport activity: whereas AcrAB-TolC has a clear role in detoxification by transporting out antibiotics and other noxious products, MmpL3 has a major role as both a trehalose monomycolate (TMM) transporter (hence contributing to the assembly of mycobacterial cell envelope) and a heme importer [34, 35, 42]. It is tempting to believe that inhibiting a protein involved in two such different and important pathways is like having found the Achilles' heel of Mtb. In addition, since several of these MmpL3 inhibitors act by dissipating the proton gradient, which is the energy source used by MmpL3 itself and many other transport proteins [63, 64], it is expected that lipid transport in general (not only TMM transport) would be greatly affected, given the implication of other MmpL proteins in the transport of other essential lipids (PDIM in the case of MmpL7, sulfolipid-1 in the case of MmpL8, acylated trehaloses in the case of MmpL10, and monomeromycolyl diacylglycerol in the case of MmpL11).

In summary, MmpL proteins have emerged also as attractive drug targets in Mtb, given their pivotal role in lipid transport (and other essential processes) in this pathogen. Including specifically MmpL proteins in drug discovery programmes could be challenging, given the intrinsic difficulty to work with membrane proteins, but this should be overcome by the expectations of finding a new effective drug candidate against tuberculosis.

FOOTNOTES

Conflicts of interest

The authors declare no conflicts of interest.

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LEGENDS TO TABLES & FIGURE

Table 1

Drugs used in the treatment of tuberculosis.

Table 2

The commonly used antituberculosis drugs with the genes associated with their respective resistance and major mechanism.

Table 3

MmpL and MmpS proteins of *Mycobacterium tuberculosis* H37Rv

Table 4

Inhibitors of MmpL3

Figure 1

Cell envelope of *Mycobacterium tuberculosis*.

Figure 1 color

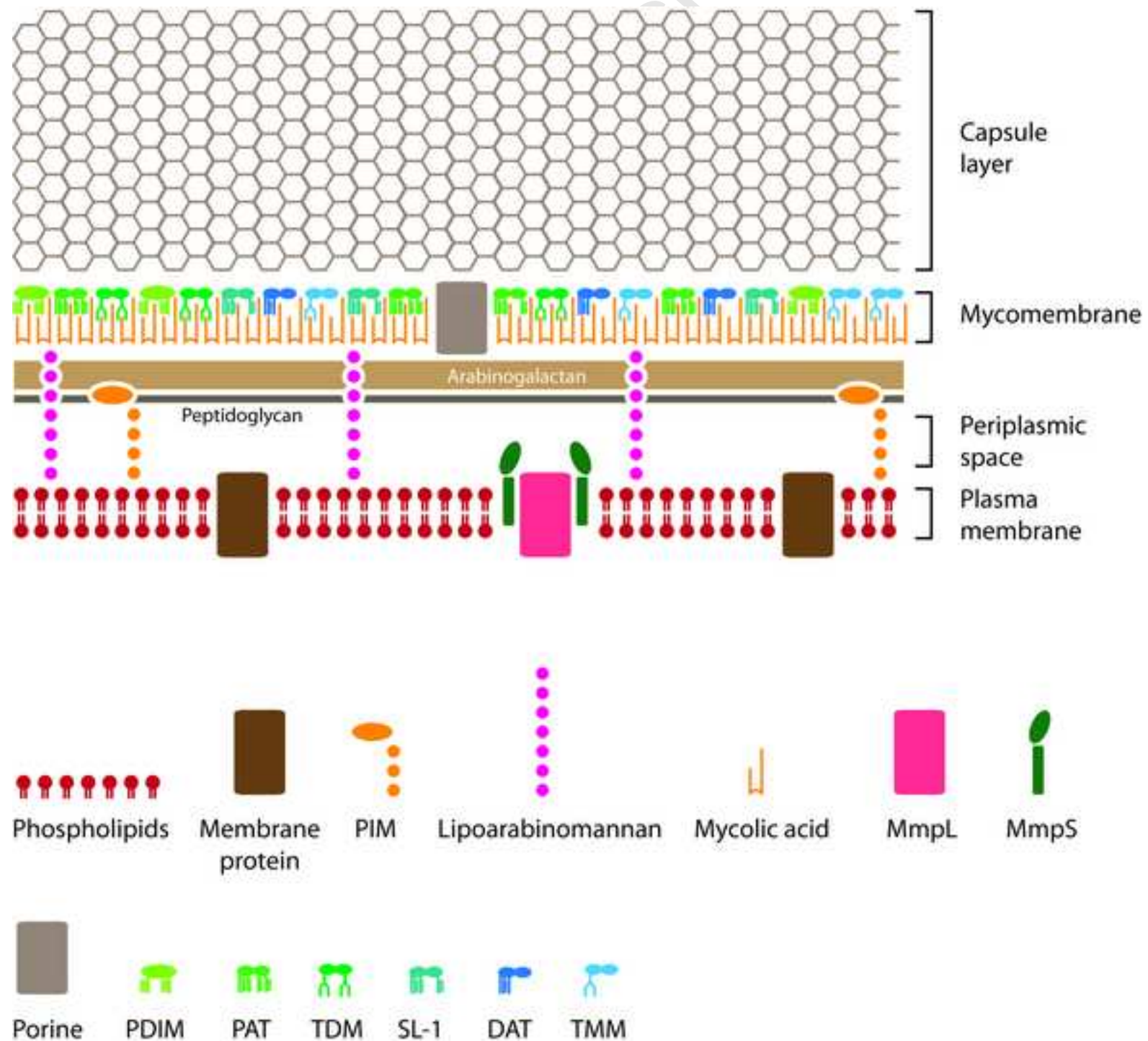
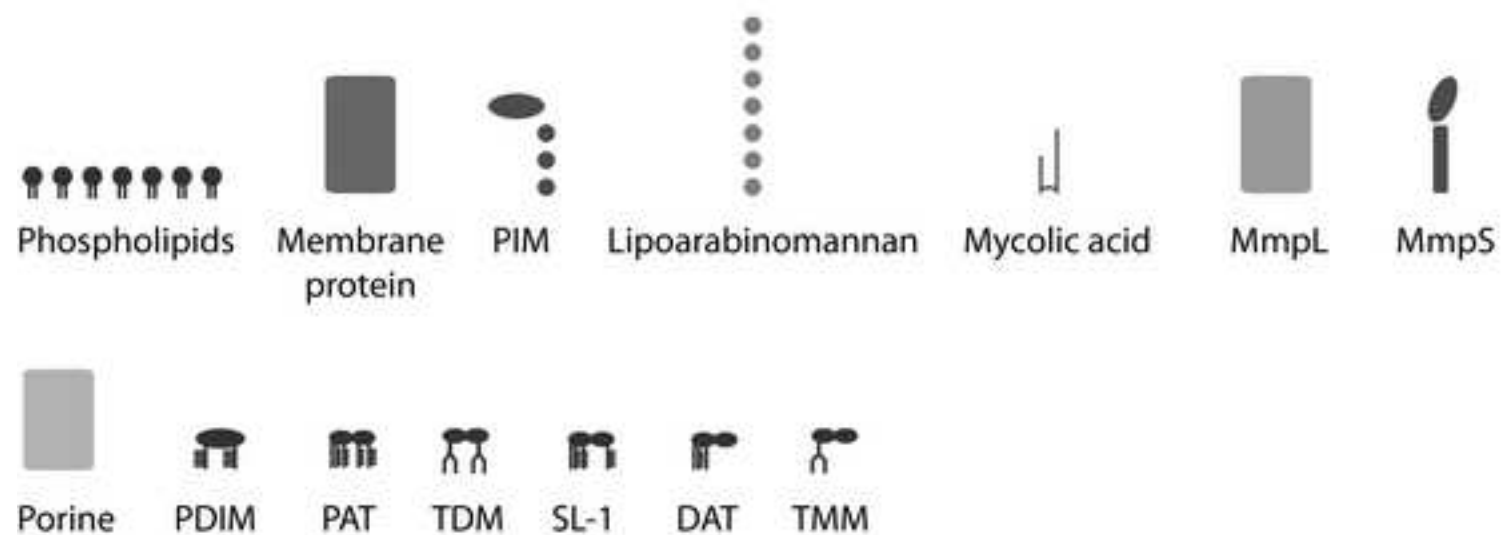
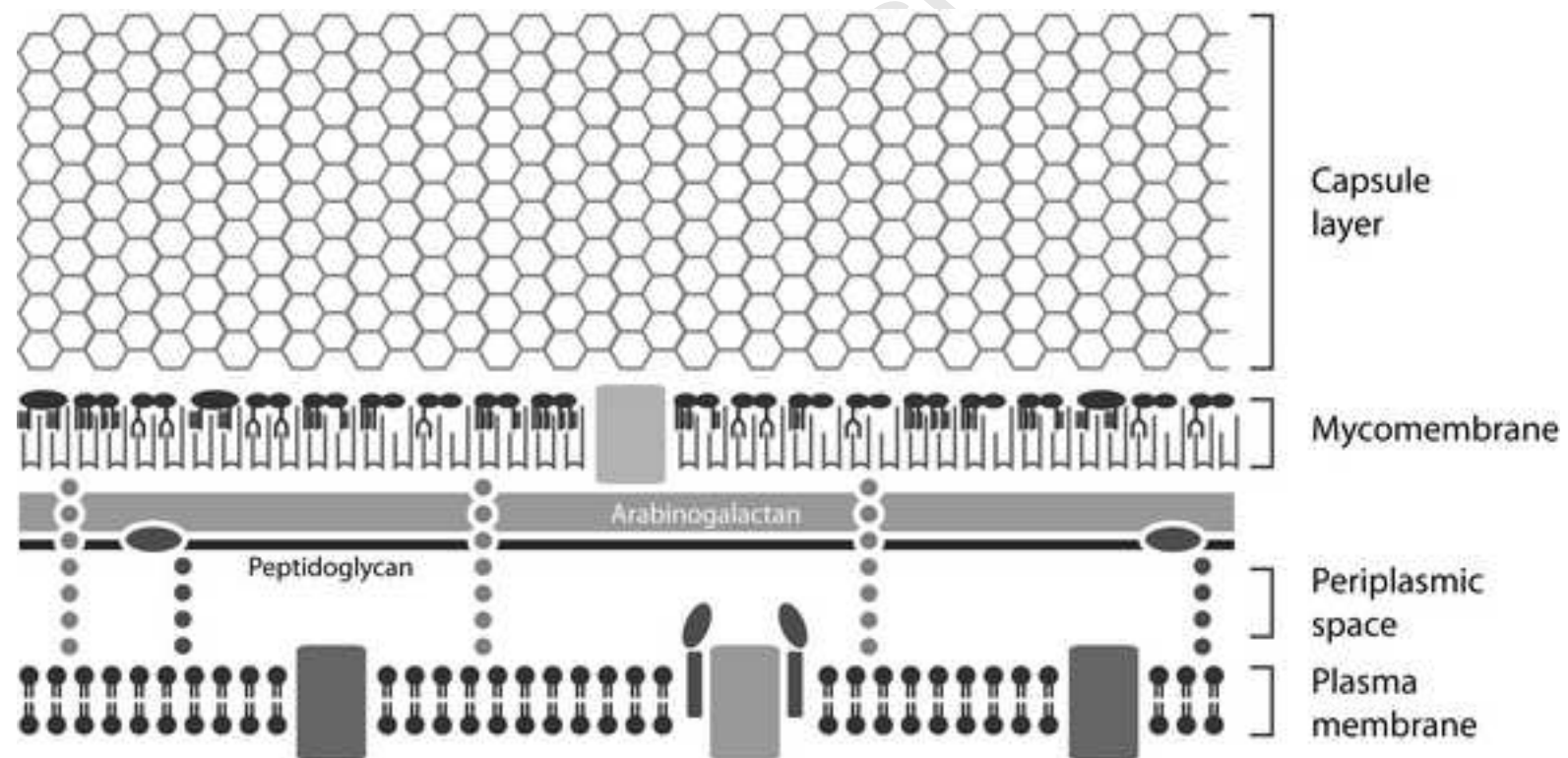


Figure 1 black&white



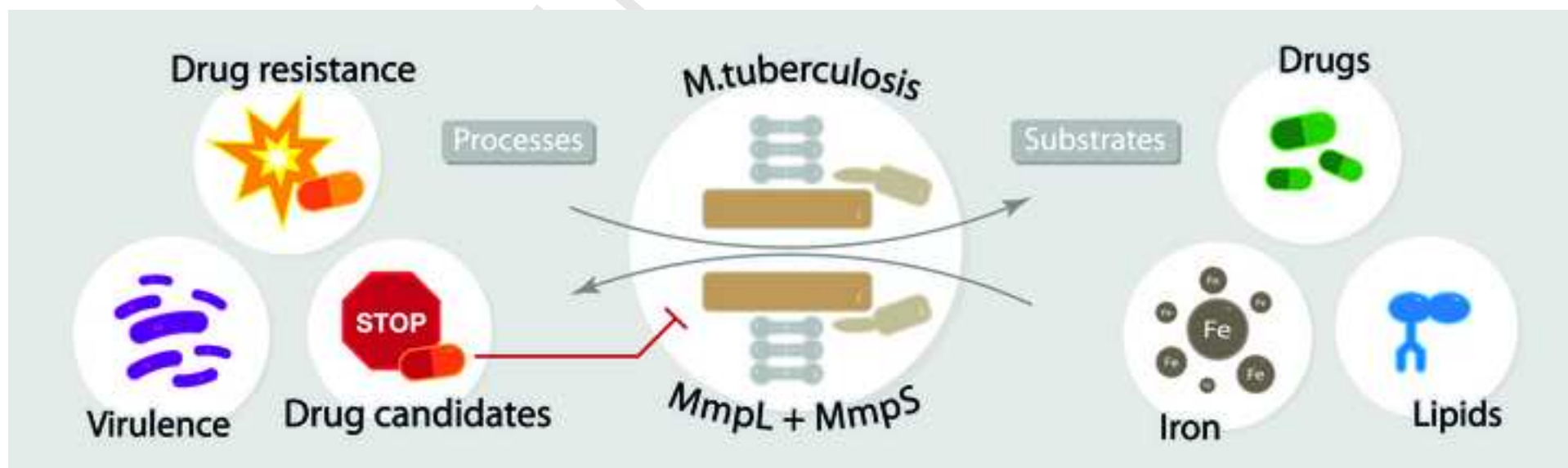


Table 1. Drugs used in the treatment of tuberculosis.

Groups of drugs	Drugs
First-line drugs	Isoniazid Rifampicin Pyrazinamide Ethambutol Rifapentine Rifabutin
Second-line drugs	Aminoglycosides (Streptomycin, Kanamycin, Amikacin) Polypeptides (Capreomycin, Viomycin) Fluoroquinolones (Ciprofloxacin, Levofloxacin, Moxifloxacin, Ofloxacin, Gatifloxacin) Para-aminosalicylic acid Cycloserine Terizidone Ethionamide Prothionamide Thioacetazone Linezolid
Third-line drugs	Clofazimine Linezolid Amoxicillin plus clavulanate Imipenem plus cilastatin Clarithromycin

Table 2. The commonly used antituberculosis drugs with the genes associated with their respective resistance and major mechanism.

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Drug or drug class	Mechanism of action	Genes associated with drug resistance	Gene number*	Protein function	Mechanism of drug resistance
Rifamycins (rifampicin, rifabutin, etc.)	Inhibition of RNA synthesis	<i>rpoB</i>	Rv0667	RNA polymerase β -subunit	Target modification
Isoniazid	Inhibition of mycolic acid biosynthesis and multiple effects in DNA, lipids, carbohydrates, and NAD metabolism	<i>katG</i>	Rv1908c	Catalase- peroxidase enzyme	Decreased drug activation
		<i>inhA</i>	Rv1484	NADH-dependent enoyl-acyl carrier protein	Target amplification or modification
Pyrazinamide	Depletion of membrane energy and other effects	<i>pncA</i>	Rv2043c	Pyrazinamidase	Decreased drug activation
Ethambutol	Inhibition of arabinogalactan synthesis	<i>embCAB</i>	Rv3793-5	Arabinosyltransferases	Target modification
Streptomycin	Inhibition of protein synthesis	<i>rpsL</i>	Rv0682	12S ribosomal protein	Target modification
		<i>rrs</i>	MTB000019	16S rRNA	Target modification
		<i>gidB</i>	Rv3919c	7-Methylguanosine methyltransferase	Target modification
Kanamycin/amikacin	Inhibition of protein synthesis	<i>rrs</i>	MTB000019	16S rRNA	Target modification
		<i>eis</i>	Rv2416c	Aminoglycoside acetyltransferase	Increased drug inactivation
Capreomycin	Inhibition of protein synthesis	<i>rrs</i>	MTB000019	16S rRNA	Target modification
		<i>tlyA</i>	Rv1694	rRNA methyltransferase	Target modification
Quinolones	Inhibition of DNA gyrase	<i>gyrA</i>	Rv0006	DNA gyrase A	Target modification
		<i>gyrB</i>	Rv0005	DNA gyrase B	Target modification

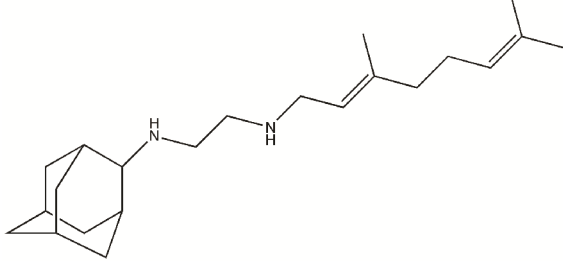
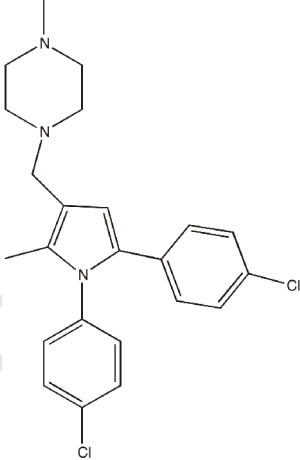
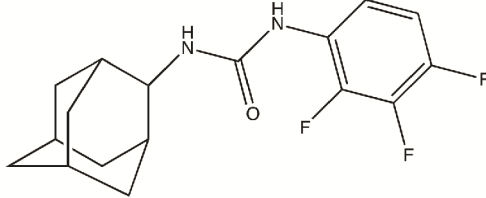
Ethionamide	Inhibition of mycolic acid synthesis	<i>ethA</i>	Rv3854c	Mono-oxygenase	Decreased drug activation
		<i>ethR</i>	Rv3855	Transcriptional regulatory repressor protein (TetR family)	Decreased drug activation
		<i>inhA</i>	Rv1484	NADH-dependent enoyl-acyl carrier protein	Target amplification and modification
Para-aminosalicylic acid	Inhibition of folic acid and iron metabolism	<i>thyA</i>	Rv2764c	Thymidylate synthase	
		<i>ribD</i>	Rv2671	Enzyme in riboflavin biosynthesis	
Cycloserine	Peptidoglycan biosynthesis	<i>alr</i>	Rv3423c	Alanine racemase	
		<i>ddl</i>	Rv2981c	D-Alanine-D-alanine ligase	
		<i>cycA</i>	Rv1704c	Bacterial D-serine/L- and D-alanine/glycine/D-cycloserine proton symporter	
Bedaquiline	Inhibition of ATP synthesis	<i>atpE</i>	Rv1305	ATP synthase	Target modification
Linezolid	Inhibition of protein synthesis	<i>rrl</i>	MTB000020	23S rRNA	Target modification
		<i>rplC</i>	Rv0701	50S ribosomal protein L3	Target modification

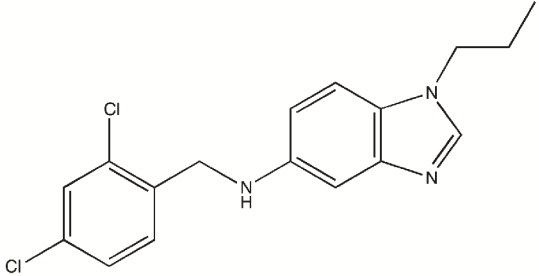
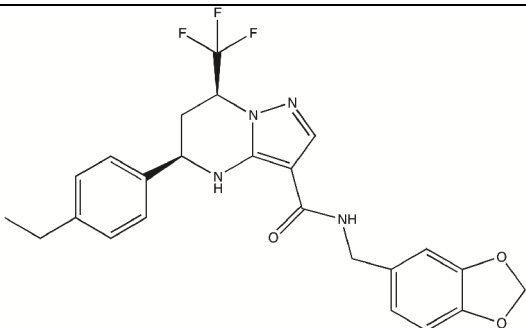
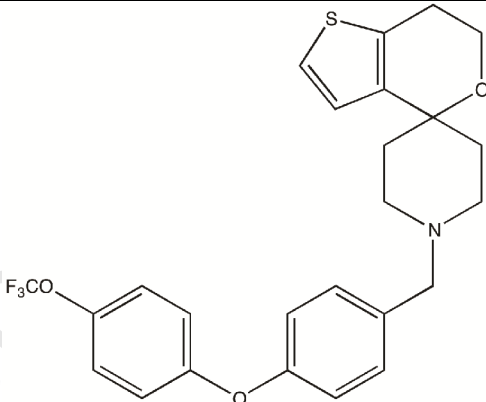
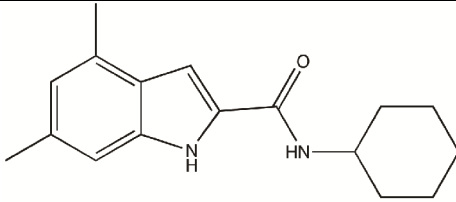
*: Gene numbers are given according to Tuberculist database (<http://tuberculist.epfl.ch/>)

Table 3: MmpL and MmpS proteins of *Mycobacterium tuberculosis* H37Rv

Name	Rv number	Protein length
		(amino acids)
MmpL1	Rv0402c	958
MmpL2	Rv0507	968
MmpL3	Rv0206c	944
MmpL4	Rv0450c	967
MmpL5	Rv0676c	964
MmpL6	Rv1557	397
MmpL7	Rv2942	920
MmpL8	Rv3823c	1089
MmpL9	Rv2339	962
MmpL10	Rv1183	1002
MmpL11	Rv0202c	966
MmpL12	Rv1522c	1146
MmpL13a	Rv1145	303
MmpL13b	Rv1146	470
MmpS1	Rv0403c	142
MmpS2	Rv0506	147
MmpS3	Rv2198c	299
MmpS4	Rv0451c	140
MmpS5	Rv0677c	142

Table 4: Inhibitors of MmpL3

Drug	Structure	References
SQ109		[54]
1,5-diarylpyrrole derivatives (BM212)		[55, 58]
adamantyl ureas (AU1235)		[34, 57, 61]

benzimidazole (C215)		[62]
tetrahydropyrazolo[1,5-a]pyrimidine-3-carboxamides (THPPs)		[60]
N-benzyl-6',7'-dihydrospiro[piperidine-4,4'-thieno[3,2-c]pyrans		[60]
indolecarboxamides		[56, 59]